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Review Article

Microbiological Features of Solid State Fermentation and its Applications - An overview

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Solid state fermentation [SSF] has been recently considered as the most cheapest and more environmentally friendly relative to submerged liquid fermentation [SLF] in the production of value added industrial based products such as enzymes, bio fuels and the likes. The merit and demerit of SSF and SLF has been summarized. The main microorganisms that occupied a pivotal position in achieving absolute SSF processes have been highlighted. A typical process and production characteristic of a selected bioreactor has been addressed within the concept of SSF. The applications of the process in various economic sectors such as industrial fermentation, agro food industry and environmental control have been reported. This review aimed at gathering the disperse literature on the current state of art on SSF, its advantages and disadvantages relative to SLF and their various applications.

Keywords: Applications, bioreactor, microbiological, SLF and SSF.

Solid State Fermentation

In contrast to Submerged (liquid state) Fermentation, Solid State Fermentation (SSF) is the growth and/or Cultivation of micro organisms under controlled conditions in the absence of free water for the production of desired products of interest. Examples of products of Solid State Fermentation include industrial enzymes, fuels and nutrient enriched animal feeds. The application of modern biotechnical knowledge and process control technologies can extremely lead to significant productivity increases from this ancient process.

Comparative studies between submerged liquid fermentation (SLF) and SSF have proved higher yields and other advantages for products made by SSF [1-6]:

- The low availability of water reduces the possibilities of contamination by bacteria and yeast. This allows working in aseptic conditions in some cases.
- Higher levels of aeration, especially adequate in those processes demanding an intensive oxidative metabolism.
- Similar environment conditions to those of the natural habitats for fungi, which constitute the main group of microorganisms used in SSF.
- The inoculation with spores (in those processes that involve fungi) facilitates its uniform dispersion through the medium.
- The substrate usually provides all the nutrients necessary for growth. Therefore culture medium composition is often quite simple.
- Reactors with simple design and few spatial requirements can be used due to the concentrated nature of the substrates.

- SSF is in most cases characterized with low energy requirement which may likely reduces the production cost at industrial level as autoclaving or vapour treatment, mechanical agitation and aeration are not often necessary in some cases.
- Polluting effluents volumes are generally small. Fewer requirements of dissolvent is evident for product extraction due to their high concentration.
- The peculiar feature of low moisture availability may facilitate the production of specific compounds that may not likely be produced or poorly produced in SLF
- The product obtained in SSF have slightly different desired properties i.e more thermotolerance relative to their counterparts obtained in SLF.
- Some SSF bioreactors have easier downstream processing

Despite the aforementioned advantages of SSF over SLF, SSF is beset with following disadvantages as reported by [1-6]:

- The substrate in most cases requires pretreatment which include size reduction by grinding, physical or chemicaland enzymatic hydrolysis, cooking or vapour treatment.
- Microorganisms like bacteria which may require high moisture levels can perform poorly in SSF. Therefore fungi perform better than bacteria, because of its low moisture requirement.
- Difficulties are usually encountered in biomass determination.
- Monitoring of process parameter such as pH, moisture content, substrate, oxygen and biomass concentration becomes a problem because of solid nature of the substrate.
- Static condition is mostly preferred as agitation most often proved to be very difficult.
- The engineering and some scientific characterization of SSF bioreactors is not yet fully matured as such there are scarcity of information about the design and operation of reactors on a large scale.
- There is possibility of contamination with unwanted fungal species.
- Aeration may be difficult sometimes due to high solid concentrations
- Spores needs to be germinated as they usually have longer lag phases, so cultivation times are longer than in SLF.

Despite the disadvantages of SSF, scientist still believes that is going to solve many of the present industrial production and some of the environmental plights. Some researchers developed Terrafors 15L bioreactor, MADEP SA designed for thermophilic xylanase production process using previously isolated bacteria strains [7]. Table 1 and 2 below summarizes the typical process characteristics of the aforementioned bioreactor.

Table 1. Typical process characteristics of Terrafors 15L bioreactor, MADEP SA

Solid Matrix	Rice bran, sawdust, sugar beet pulp, etc
Liquid Medium	Basic Mineral Medium
Inoculation	1 to 20% (w/w) of fungi or bacteria suspension
Temperature	5 to 95 C
Moisture content	50 to 85%
Agitation	Intermittent or constant rotation
Aeration	0.5 to 5 vessel volumes per hour
Duration	1 to 5 days
Downstream processing	None or centrifugation for many applications

The major objective of the research group is to Produce pure cultures of thermophilic xylanase secreting bacteria and develop a production process for thermophilic xylanase enzymes. Applications of thermophilic xylanase enzymes include waste paper de-inking, biopulping and fuel ethanol production from lignocellulosic materials.

Table 2. Product and production process characteristics (15 liter bioreactors, MADEP SA)

Microbial source	Natural (not genetically modified) bacteria strain
Production process	Solid state fermentation
Volumetric productivity	8 times greater than submerged fermentation productivity (submerged fermentation with pure xylan inducer (expensive) and SSF **without** inducer). The only source of xylan in the SSF process is the solid matrix material (a low cost food processing waste product).
Formulation	Crude fermentation product (bacteria, enzymes, water and solid matrix) or crude enzyme extract in basic mineral medium.
Enzyme pH optimum	pH 6
Enzyme temperature optimum	75^0 C
Cellulase activity	< 10% by DNS assay using CMC as substrate
Microbial purity	100% at the end of fermentation

Microorganisms

Bacteria, yeasts and fungi can grow on solid substrates, and find application in SSF processes. Filamentous fungi are the best adapted for SSF and dominate in research works. Some examples of SSF processes for each category of micro-organisms are reported in table 3**.** Bacteria are mainly involved in composting, ensiling and some food processes [8]). Yeasts can be used for ethanol and food or feed production [9] . But filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties. The hyphal mode of fungal growth and their good tolerance to low water activity (A_w) and high osmotic pressure conditions make fungi efficient and competitive in natural microflora for bioconversion of solid substrates. *Koji* and *Tempeh* are the two most important applications of SSF with filamentous fungi. *Aspergillus oryzae* is grown on wheat bran and soybean for "*Koji*" production, which is the first step of soy sauce or citric acid fermentation. Koji is a concentrated hydrolytic enzyme medium required in further steps of the fermentation process. "Tempeh" is an Indonesian fermented food produced by the growth of *Rhizopus oligosporus* on soybeans. People consume the fermented product after cooking or toasting. The fungal fermentation allows better nutritive quality and degrades some antinutritional compounds contained in the crude soybean. The hyphal mode of growth gives a major advantage to filamentous fungi over unicellular microorganisms in the colonisation of solid substrates and for the utilisation of available nutrients. The basic mode of fungal growth is a combination of apical extension of hyphal tips and the generation of new hyphal tips through branching. An important feature is that, although extension occurs only at the tip at a linear and constant rate, the frequency of branching makes the kinetic growth pattern of biomass exponential, mainly in the first steps of the vegetative stage [2]. The hyphal mode of growth gives the filamentous fungi the power to penetrate into the solid substrates. The cell wall structure attached to the tip and the branching of the mycelium ensure a firm and solid structure. The hydrolytic enzymes are excreted at the hyphal tip, without large dilution like in the case of LSF, what makes the action of hydrolytic enzymes very efficient and allows penetration into most solid substrates. Penetration increases the accessibility of all available nutrients within particles [2].

Table 3. Main groups of microorganisms involved in SSF [2]

Microflora	*SSF Process*
Bacteria	
Clostidrium sp.	*Ensiling, Food*
Lactobacillus sp.	*Ensiling, Food*
Streptoccus sp.	*Composting*
Pseudomonas sp.	*Composting*
Serratia sp.	*Composting*
Bacillus sp.	*Composting, Natto, amylase*
Fungi	
Altemaria sp.	*Composting*
Penicilium notatum, roquefortii	*Penicillin, Cheese*
Lentinus edodes	*Shii-take mushroom*
Pleurotus oestreatus, sajor-caju	*Mushroom*
Aspergillus niger	*Feed, Proteins, Amylase, ctric acid*
Rhizopus oligosporus	*Tempeh, soybean, amylase, lipase*
Aspergillus oryzae	*Koji, Food, citric acid*
Amylomyces rouxii	*Tape cassava, rice*
Beauveria sp., Metharizium sp.	*Biological control, Bioinsecticide*
Trichoderma sp.	*Composting Biological control, Bioinsecticide*
Phanerochaete chrysosporium	*Composting, lignin degradation*
Rhizopus sp.	*Composting. Food, enzymes, organic acids*
Mucor sp.	*Composting, Food; enzyme*
Monilia sp.	*Composting*
Fusarium sp.	*Composting. Gibberellins*
Aspergillus sp.	*Composting, Industrial, Food*
Yeast	
Endomicopsis burtonii	*Tape, cassava, rice*
Schwanniomyces castelli	*Ethanol, Amylase*
Saccharomyces cerevisiae	*Food, Ethanol*

Applications of SSF

The various economic applications of SSF offer the potential of significantly improving and raising living standards with only a low technology input requirement [10]. Several authors have reviewed the different applications of solid-state fermentation [3,11]. SSF is briefly associated with the production of traditional fermented foods such as “koji”, Indonesian “tempeh” or Indian “ragi”. SSF has also been used for the production of high added value compounds (such as enzymes, organic acids, biopesticides, biofuel and flavours). In the last years, new applications of SSF in the environmental control have been developed including bioremediation and biodegradation of hazardous compounds and the detoxification of agroindustrial residues. Table 4 shows some examples of SSF processes in economical sectors of fermentation industry, agro industry and environmental control [3,11].

Conclusions

SSF plays a significant role at laboratory level than SLF and it is often considered a cost effective process than its counterpart for the production of wide arrays of bioproducts. SSF, as reported by various authors' interms of bio productions, posses many fold-higher than those obtained in SLF. Despite its advantages it has some major drawbacks such as control of process parameters, adequate scale-up from laboratory bench to industrial level are some of its key plight. The future trend of this phenomenon may improve as a result of continuous research to scale-up the process to industrial level for efficient realizations of desired product in an eco-efficient and sustainable manner.

Table 4. Main applications of SSF in various economic sectors [12]

Economic Sector	Application	Examples
Industrial fermentation	Enzymes production	Amylases, amyloglucosidase, cellulases, proteases, pectinases, xylanases, glucoamylases
	Bioactive products	Mycotoxins, gibberellins, alkaloids, antibiotics, hormones
	Organic acid production	Citric acid, fumaric acid, itaconic acid, lactic acid
	Biofuel	Ethanol production
	Miscellaneous compounds	Pigments, biosurfactants, vitamins, Xantham
Agro-Food Industry	Biotransformation of crop residues	Traditional food fermented (Koji, sake, ragi, tempeh), protein enrichment and single cell protein production, mushrooms production.
	Food additives	Aroma compounds, dyestuffs, essential fat and organic acids
Environmental control	Bioremediation and biodegradation of hazardous compounds	Caffeinated residues, pesticides, polychlorinated biphenyls (PCBs)
	Biological detoxification of agro-industrial wastes	Coffee pulp, cassava peels, canola meal, coffee husk

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